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Comparative evaluation of two Canola germplasm under varying levels of NPK fertilizer in Layyah irrigated zones

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ABSTRACT

Canola (*Brassica napus L.)* is a vital oilseed crop globally, renowned for its high nutritional and oil content. This study aims to assess and compare the growth and yield performance of two canola germplasm, namely Punjab and Faisal, under various NPK fertilizer levels in irrigated zones. The experiment employed a factorial experiment based on a completely randomized design with eight treatments, three replications conducted twice in 2021 and 2022 at Chak no. 114 Shahpur Daurata, situated at latitude 30.9693° N and longitude 70.9428° E. The findings indicate that the optimal fertilizer rate for canola cultivation in Pakistan is dependent on the variety and level of NPK fertilizer. The recommended NPK fertilizer enhanced the plant height, number of leaves, pods, 1000-grain weight and yield of canola significantly. In terms of yield and growth parameters, the Punjab canola variety outperformed the Faisal canola variety. The highest yield was achieved in T1 treatment that received the recommended NPK dose of (100:75:30 kg/ha-^{1}). This study's outcomes are critical for sustainable agricultural intensification, which is essential to meet the growing population's food demand. This comparative evaluation of two canola germplasm under varying levels of NPK fertilizer in irrigated zones is a crucial research domain that can provide essential insights into optimal fertilizer management practices for canola cultivation in Pakistan. Therefore, the application of recommended NPK doses in conjunction with the Punjab canola variety may result in increased crop yields for local farmers, specifically under the prevailing soil and climate conditions in Shahpur Durata.

Keywords: Arid University, Brassica, Canola, Layyah, NPK doses, Oil seed crop

1. INTRODUCTION

Canola, scientifically known as *Brassica napus* L., is the third most important oilseed crop globally and its production has expanded remarkably in most of the major producing nations in recent years (FAOSTAT, 2015; USDA, 2011). According to Newkirk, (2009), canola, which is a type of rapeseed, is a beneficial source of several essential nutrients. These include available calcium, iron, manganese, selenium and many of the B vitamins. The term "canola" presently

used in Canada is being adopted in other countries to express the modified canola low in erucic acid (<2%) and glucosinolate (<30 μmol/g of a dried defatted meal) content (OGTR, 2008; Khajali and Slominski, 2012). Canola oil has been utilized in lamps since as early as 2000 BC in India and the 13th century in Europe. In addition to its use in lighting, it has also been employed in the production of food and soap (OGTR, 2008; Gómez-Campo and Prakash, 1999). Increasing world population and renewable energy policies in some countries have driven the global demand for vegetable oils, resulting in a surge in oilseed demand (FAO, 2003; Lu et al., 2011). Soybean and canola oils are the first and third most produced vegetable oils globally among the 17 commodity fats and oils. Canola oil ranks third among edible oil sources, behind soybean and palm oils (Nowlin, 1991). Canola meal is a rich source of numerous vital nutrients. Domestic canola production would reduce import costs, enhance American farm productivity and promote agricultural diversity (Starner et al., 1996).

Canola was formed through spontaneous hybridization between turnip rape (*Brassica Rapa*) and cabbage (*Brassica oleracea*) about 7500 years ago (Chalhoub et al., 2014). It is widely grown across different parts of the world and its cultivation in India dates back to 4000 BC. It later extended to China and Japan around 2000 years ago (Snowdon et al., 2006). The winter, semi-winter, and spring varieties of Canola have different levels of tolerance to cold and drought, which influences their optimal growing conditions. Canola grows best in well-drained soils with a pH range of 5.5 to 8.5. The plant requires 110 to 150 days to attain full maturity and can grow to a height of 120 to 150 cm. The mature seeds of Canola have a spherical shape, a diameter of 1.8-2.7 mm and a red-brown to dark brown or black color (Raboanatahiry et al., 2021; Gulden et al., 2008). Canola oil is composed mainly of triacylglycerol and has a low content of saturated fatty acids compared to other common cooking oils, such as peanut (17%), olive and soybean (15%), corn (13%) and sunflower (12%) (Canola Info, 2016).

Nitrogen (N), phosphorus (P) and potassium (K) are essential macronutrients for canola growth and development. Optimal utilization of these nutrients can increase the yield of canola and reduce production costs. Canola, which belongs to the Brassica oilseed crop group, is the second most produced oilseed crop worldwide. *Brassica napus* is the predominant crop within this group, while other crops such as *Brassica juncea, Brassica rapa, Brassica carinata* and *Brassica nigra* are cultivated in specific regions globally. The rising demand for vegetable oils and the necessity for crop diversification may lead to an expansion in canola acreage in the western United States, where saline-prone soils exist (Mc-Vetty and Duncan, 2016; Francois, 1994; Eskin and Przybylski, 2003).

Canola (*Brassica napus* L.) is a globally important oilseed crop, valued for its oil and meal production. Proper nutrient management practices, particularly with respect to nitrogen (N), phosphorus (P) and potassium (K), significantly affect canola yield. N is especially crucial for canola growth and development, particularly in newly reclaimed sandy saline soils, which are deficient in organic and mineral nitrogen. Studies by Subrahmaniyan et al., (2001) and Nagavani et al., (2001) have shown that increasing NPK levels by up to 150% leads to better yield attributes and overall yield. Similarly, research by Abo El-Hamd, (2003) and Atalla, (2007) demonstrated that increasing N fertilizer levels up to 60 kg N/fed significantly increased plant height, number of pods/plant, seeds weight/pod, 1000-seed weight and seed yield per plant, while oil percentage responded to lower levels of N fertilizer, i.e., 20 kg N/fed. P is another vital nutrient component in canola management practices as it is a critical constituent of the genetic material of the cell nucleus in canola. Cells cannot divide unless there is sufficient P to form new nuclei and P deficiency can delay maturity and reduce seed quality, ultimately affecting vegetative growth. Research by Aulakh and Parsicha, (1999) has demonstrated that mustard crops respond significantly (46%) to P fertilizer rates up to 40 kg P2O5/ha (Jayesh et al., 2000) found that direct application of P at the rate of 60 kg P2O5/ha resulted in higher yields of gobhi sarson (canola). K is another essential nutrient that plays a critical role in canola growth and development. It is involved in critical processes like photosynthesis, protein synthesis and assimilates translocation. According to Khan et al., (2004), increasing K fertilizer levels from 0 to 25, 75, 100, 125 and 150 kg K2O/ha significantly increased seed yield, number of seeds per pod and 1000-seed weight. However, the highest value of oil content was obtained from the control treatment (no fertilizer).

The objective of the twice-year 2021 and 2022 research is to assess and contrast the growth and yield performance of two canola germplasm subjected to different levels of NPK fertilizer in irrigated zones. The study intends to determine the optimal fertilizer dose for canola cultivation in Pakistan, which can improve its productivity and profitability. The results of this investigation can also function as a guideline for farmers to enhance their fertilizer management practices, thus benefiting the economy of the country. This comparative evaluation of two canola germplasms under varying levels of NPK fertilizer in irrigated zones is a critical research area that can provide significant insights into the optimal fertilizer management practices for canola cultivation in Pakistan. The outcomes of this study can contribute to the sustainable intensification of agriculture, which is essential for meeting the growing population's food demand.

2. MATERIALS AND METHOD

The experiment was conducted at Layyah Chak no. 114 Shahpur Daurata, located at latitude 30.9693° N and longitude 70.9428° E. The total area of the experiment was 544 square feet (equivalent to 2 Marla), with a plot width of 17 feet and a plot length of 16 feet for one variety means while each variety contain Marla. The experiment working a factorial experiment based on a complete randomized design with eight treatments, three replications conducted for each variety (Punjab Canola and Faisal Canola). Each treatment had dimensions of 17 ft x 3 ft. There was a 4 feet gap between the two varieties. The treatment-to-treatment distance was 30 cm; replication to replication distance was 6cm, while the plant-to-plant distance was maintained at 4 cm.

The experiment was conducted twice, once in 2021 and again in 2022. The same layout was followed in both years, with only the sowing date and harvest date differing. The sowing date for the 2021 experiment was 27th September, while the harvest was conducted on 28th February of the following year. For the 2022 experiment, the sowing date was 30th September and the harvest was conducted on 8th March of the following year. The seedbed was prepared through three ploughing operations and one planker operation. The recommended seed rate of 4 kg/ha-1 was followed and the seed depth was maintained at 2-3 cm. The irrigation schedule consisted of 4 irrigations, with the first irrigation conducted 35 days after germination, the second irrigation at the bud formation stage, the third irrigation at the time of flowering and the fourth irrigation at the time of seed filling. Four different treatments were applied, with the recommended NPK doses varying for each treatment. For T1, the recommended NPK dose was (100:75:30 kg/ha-^{1}), which was equivalent to 250:188:75 g/Marla. For T2, the recommended NPK dose was 80:50:20kg/ha-^{1}, which was equivalent to (200:125:50g/Marla). For T3, the recommended NPK dose was (60:35:10 kg/ha-^{1}), which was equivalent to (150:88:25 g/Marla). The NPK fertilizers were applied by flooded method in three equal split doses; first at the time of sowing, second at the time of flowering and third at the time of seed filling, the control treatment, received no NPK fertilizer application. Soil analysis was conducted prior to the start of the experiment to determine the initial nutrient status of the soil. The soil was found to have 40.71% sand, 35.30% silt and 23.99% clay, with a pH of 7.8 and an electrical conductivity (EC) of 1.4 dSm-^{1}. The organic matters content of the soil was found to be 0.87% showed in (Table 3). The soil analysis data was obtained from the soil science laboratory of Layyah district using a self-provided soil sample.

Table 1 Recommended Fertilizer Doses and Applied Amounts of DAP, Urea and SOP

Conversion into fertilizers amount					
Treatments	Recommended Doses	Applied NPK doses	DAP	Urea	SOP
T_1	(NPK 100:75:30 kg/ha-^{1})	(NPK 250:188:75 g/marla)	408 g	475g	150g
T_2	(NPK 80:50:20 kg/ha-^{1})	(NPK 200:125:50 g/marla)	271g	385g	00g
T_3	(NPK 60:35:10 kg/ha-^{1})	(NPK 150:88:25 g/marla)	191g	295g	50g
T_4	No fertilizer applied				

Table 2 Strategic Fertilizer Application Timing and Precise Fertilizer Amounts

Doses of NPK			
Stages	T1	T2	T3
Sowing	158:136:50g	128:90:33g	98:63:16 g
Flowering	158:136:50g	128:90:33g	98:63:16 g
Pod filling	158:136:50g	128:90:33g	98:63:16 g

Table 3 Parameter Quantity of Soil

Sand	40.71%
Silt	35.30
Clay	23.99%
PH	7.8
EC	1.4 dSm-1
Organic matter	0.87%

Table 3 presents the measured values of various parameters related to soil composition. The first parameter listed is "Sand" with a value of 40.71%, followed by "Silt" with a value of 35.30%. The third parameter is "Clay" with a value of 23.99%. The next

parameter is "PH" with a value of 7.8, indicating that the soil is slightly alkaline. The "EC" or electrical conductivity of the soil is 1.4 dSm^{-1}. Finally, the table lists the percentage of "Organic matter" in the soil, with a value of 0.87%. These parameters are crucial for understanding the physical and chemical properties of the soil and can inform decisions related to land use, crop management and soil conservation practices

The labor-intensive process of traditional canola harvesting and threshing by hand involves manually cutting the plants and stacking them into bundles or sheaves, which are then threshed to separate the grain from the straw. The following parameters were measured in both years of the experiment: Plant height, number of leaves and number of pods, 1000-grain weight and yield. Plant height was measured from the base of the stem to the tip of the plant using a measuring tape. The number of leaves per plant was counted by visually inspecting each plant and counting the total number of leaves. The number of pods per plant was determined by counting the total number of mature pods on each plant. For 1000-grain weight, a sample of 1000 seeds were collected from each plot and weighed using an electronic balance. The average weight of the seeds was calculated and reported as 1000-grain weight. Finally, the yield was calculated by harvesting the plants from each plot and threshing the seeds. The yield data will be recorded in kg per Marla and then converted to kg per hectare. The results will be analyzed and compared for each treatment and variety. Based on the findings, recommendations will be made to the farmers regarding the most effective fertilizer regime for each variety in the irrigated zones of Layyah Chak no. 114 Shahpur Daurata.

3. RESULTS

The study aimed to investigate the impact of different NPK doses on the growth and yield of canola plants, as well as compare the performance of two canola varieties (V1 and V2). Four treatments were used, including T1 (NPK 250:188:75 g/Marla), T2 (NPK 200:125:50 g/Marla), T3 (NPK 150:88:25 g/Marla) and T4 with no NPK. The Table 4 results showed that treatment T1 had the most positive effect on plant growth and yield. The study found that V1 had a higher yield potential compared to V2 under the given conditions.

Plant Height

The results of the study showed that the mean plant height was the highest in the treatment with the highest NPK dose (250:188:75 g/Marla) and gradually decreased with decreasing doses of treatment. The variety V1 also had a significantly higher plant height than V2 in 2021 observed in Figure 2 and also V1 significantly higher plant height in 2022 (Figure 4). Plant height is a crucial factor as it affects the canopy development, light interception and ultimately the yield by influencing the photosynthesis rates.

Plant Population

The study found that the mean plant population was the highest in treatment T1 in 2021 (Figure 1) same trend followed in 2022 (Figure 3) investigate the trend and decreased gradually with decreasing doses of treatment. The variety V1 also had a significantly higher plant population than V2. Plant population is an important factor as it directly impacts the overall yield of the crop. However, a higher plant population may lead to a higher yield only if the plants have sufficient resources for proper growth and development.

Number of Pods per Plant

The number of pods per plant was the highest in treatment T1 and decreased with decreasing doses of treatment (Figure 6). The variety V1 also had a significantly higher number of pods per plant compared to V2 (Figure 5). The number of pods per plant is a critical yield component as it directly affects the number of seeds produced per plant, which ultimately influences the overall yield.

1000-Grain Weight

The study found that the 1000-grain weight was the highest in treatment T1 and decreased with decreasing doses of treatment. The variety V2 had a significantly higher 1000-grain weight than V1 (Figure 2, 4) investigate V2 showed significant results. The 1000-grain weight is important as it influences the yield per unit weight of the crop, which can affect the transportation and storage costs of the crop.

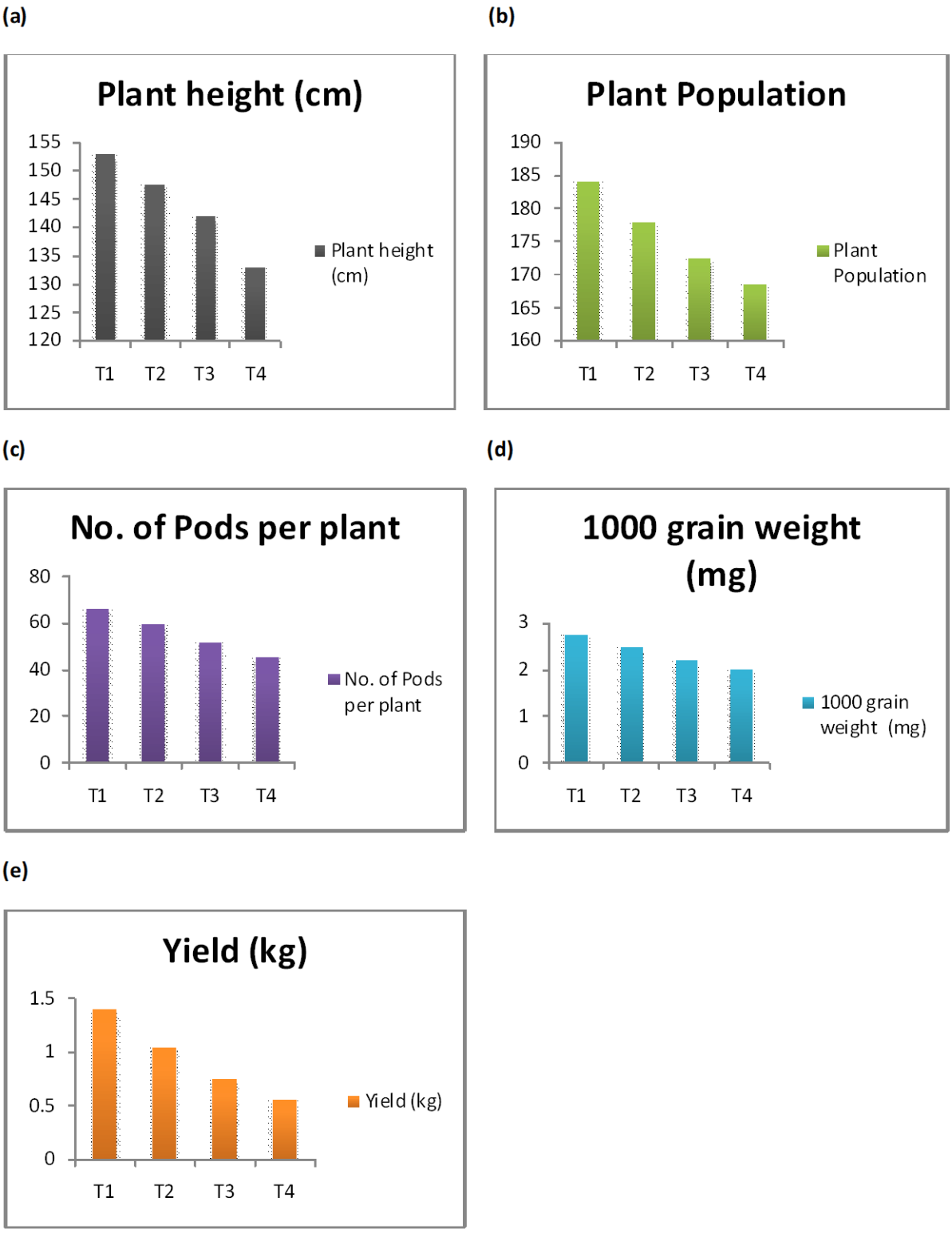

Figure 1 Comparative Analysis of Parameter Variations among NPK Treatments with Varied Doses in 2021

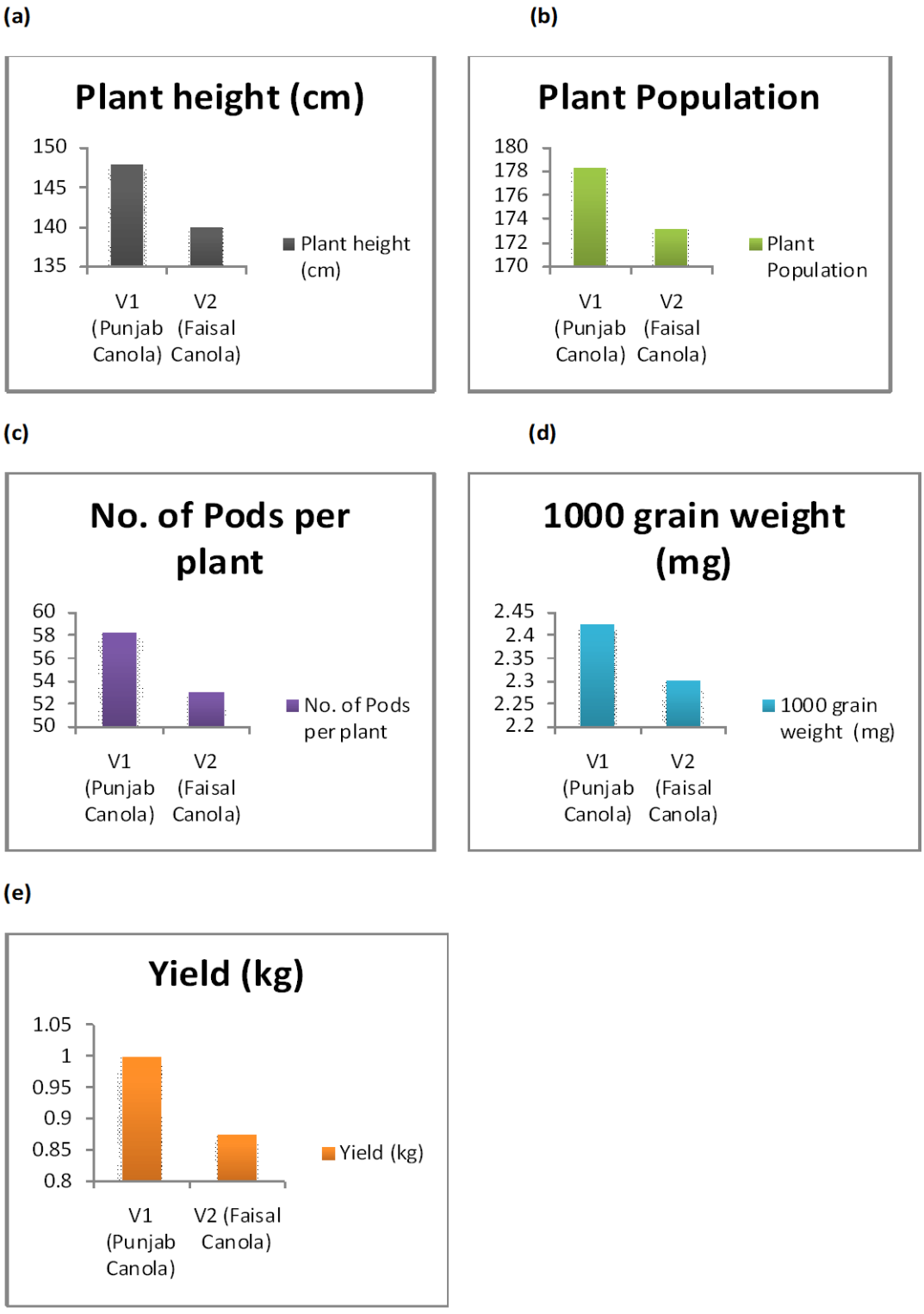

Figure 2 Effect of Varied NPK Doses on the Performance of Different Varieties in 2021

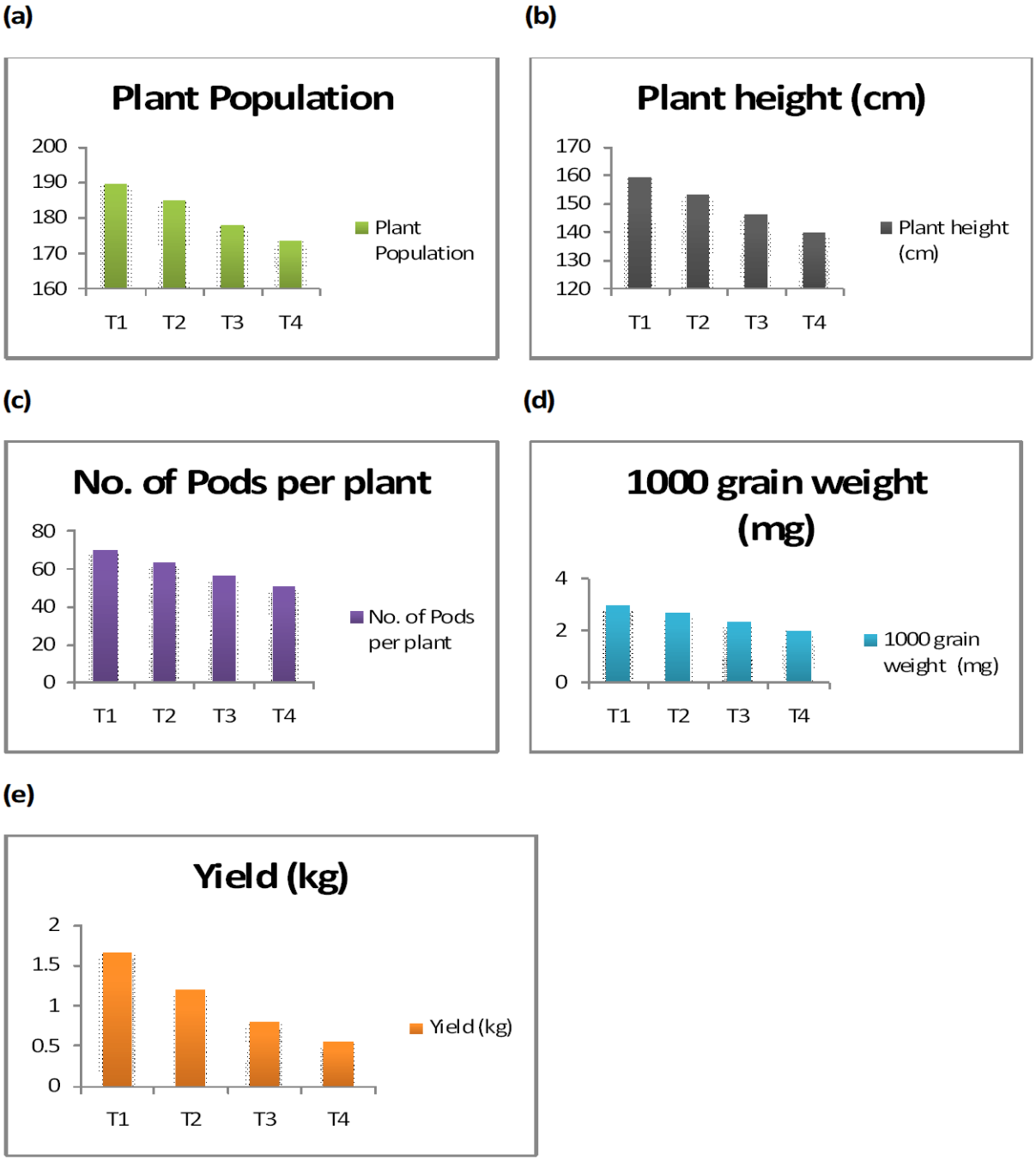

Figure 3 Exploring the Impact of Varied NPK Doses on Treatment and Parameters performance in the 2022 Crop

Yield

The study found that the yield was the highest in treatment T1 and decreased with decreasing doses of treatment observed in (Figure 1, 3). The variety V1 also had a significantly higher yield compared to V2 (Figure 5). Yield is the most important factor as it directly affects the profitability of the crop. Hence, it is crucial to optimize the NPK doses and select the right variety to obtain the maximum yield.

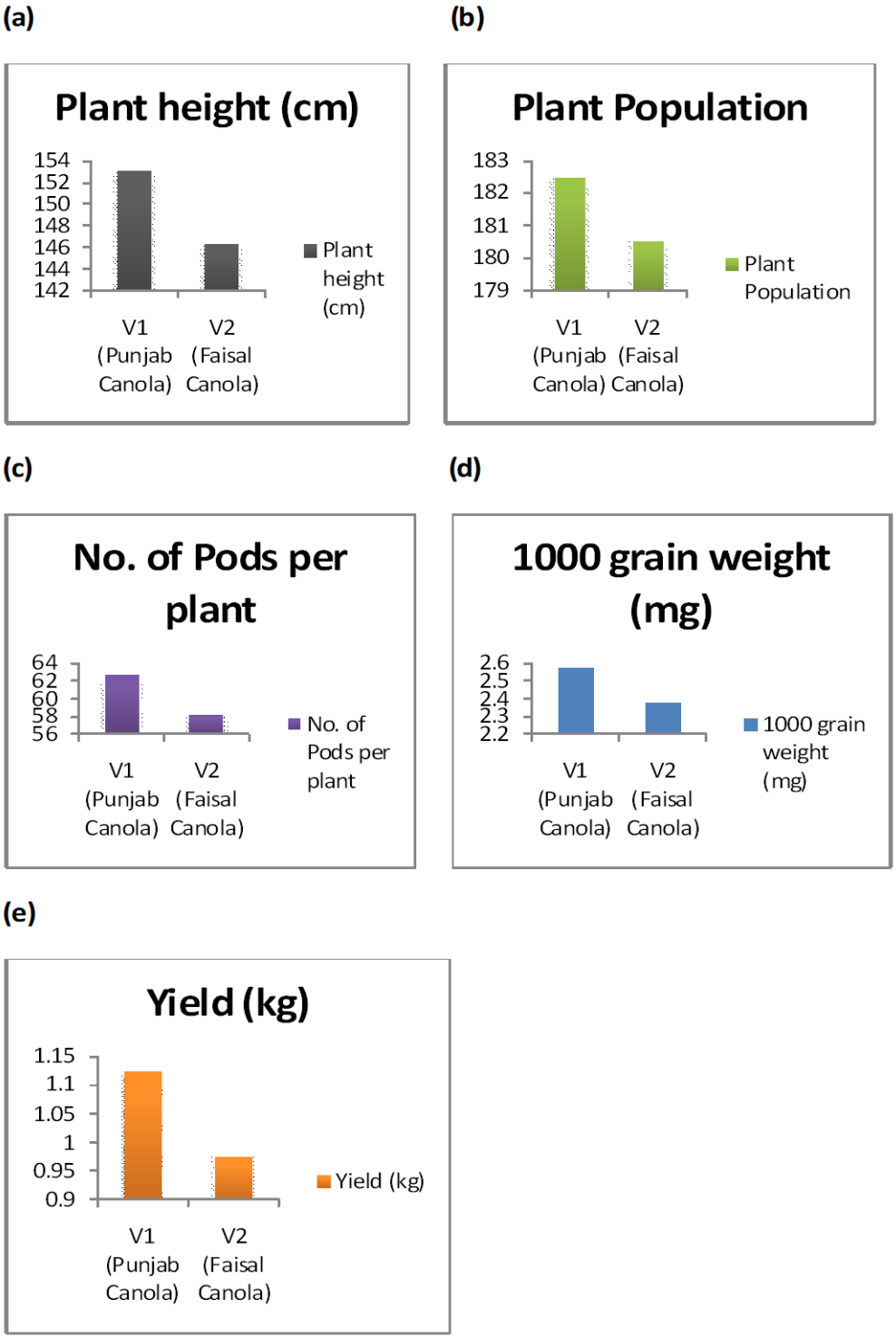

Figure 4 Varietal Performance Response to Different NPK Doses in the 2022 Crop: A Comparative Study of Performance Parameters

Table 4 Assessing the temporal stability of crop variety performance: A two-year analysis

Variety	Year	Plant height (cm)	Plant Population	No. of Pods per plant	1000 grain weight (mg)	Yield (kg)
V1	2022	150.25 a	182.50 a	62.750 a	2.5750 a	1.1250 a
V1	2021	147.75 b	180.50 a	58.250 b	2.4250 ab	1.0000 b
V2	2022	146.25 c	178.25 a	58.250 b	2.3750 b	0.9750 bc

V2	2021	140.00 d	173.25 b	53.000 c	2.3000 b	

The study concluded that treatment T1 with NPK 250:188:75 g/Marla were the most effective in promoting plant growth and yield in canola crops. The study also showed that V1 had a higher yield potential compared to V2 under the given conditions (Table 4). The findings of this study can be useful for farmers and researchers in selecting the appropriate NPK doses and canola variety for improving crop yield.

Plant Growth Response to NPK Doses

Treatment T1 with NPK 250:188:75 g/Marla produced the tallest plants, highest plant population, highest number of pods per plant, highest 1000-grain weight and highest yield. The mean plant height, plant population, number of pods per plant, 1000-grain weight and yield decreased gradually with decreasing doses of treatment. These findings suggest that treatment T1 had the most positive effect on yield components.

Table 5 A comparative study of treatment and variety effects on crop performance over two consecutive years

Treatments	Varieties	Plant height (cm)	Plant Population	No. of Pods per plant	1000 grain weight (mg)	Yield (kg)
T1	V1	158.50 a	189.00 a	71.000 a	2.9000 a	1.6000 a
T1	V2	152.50 b	184.50 ab	65.500 b	2.8000 ab	1.4500 a
T2	V1	153.00 b	184.00 ab	63.500 b	2.6500 bc	1.2000 b
T2	V2	146.50 c	179.00 bc	60.000 c	2.5000 cd	1.0500 b
T3	V1	146.50 c	176.50 cd	57.000 d	2.3500 de	0.8500 c
T3	V2	140.50 d	174.00 cd	51.500 e	2.1500 ef	0.7000 cd
T4	V1	138.00 e	172.00 d	50.500 e	2.1000 fg	0.6000 de
T4	V2	133.00 f	170.00 d	45.500 f	1.9000 g	0.5000 e

The Table 5 compares the performance of different treatments and varieties in two different years. In the treatment comparison, four treatments (T1, T2, T3 and T4) were tested on two different varieties (V1, V2) and their performance was measured in terms of plant height, plant population, number of pods per plant, 1000 grain weight and yield (Figure 5). In the variety comparison, the performance of two varieties (V1, V2) was tested in two different years (2021, 2022) and measured using the same parameters (Figure 5). Based on the treatment comparison, it can be observed that treatment T1 performed the best in all parameters for both varieties. Treatment T4 showed the worst performance for both varieties. In terms of varieties, it can be observed that V1 performed better than V2 in all parameters for both years. The results suggest that treatment T1 and variety V1 are the best performers in terms of yield and other parameters and these can be recommended for further cultivation.

Performance Comparison of Canola Varieties

The study compared the performance of two canola varieties, V1 and V2. Plant height, plant population, number of pods per plant, 1000-grain weight and yield were evaluated. The results showed (Figure 5) that V1 had significantly higher plant height, plant population, number of pods per plant and yield compared to V2. However, V2 had a significantly higher 1000-grain weight compared to V1. This finding suggests that V2 produces denser seeds, which can contribute to higher yield per unit weight.

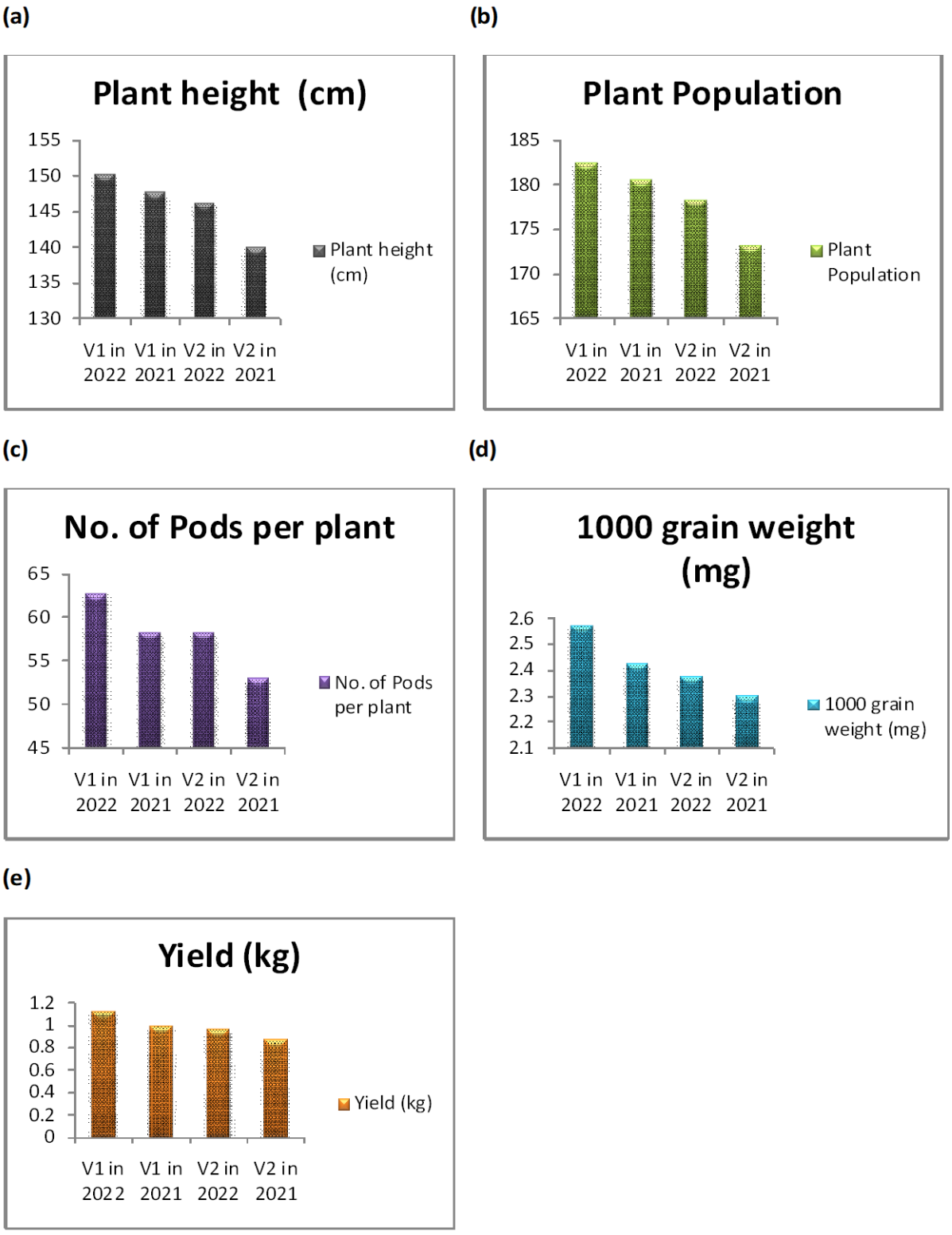

Figure 5 Integrative Analysis of Multi-Year Varietal Performance Parameters: Unraveling the Dynamics of Genotypic Expression in Agricultural Systems

Table 6 shows the yield in kilograms of two different varieties of a crop (V1 and V2) in two different years (2021 and 2022). In 2021, V1 had a higher yield of 4 kg compared to V2 which had a yield of 3.5 kg. In 2022, both varieties showed an increase in yield, with V1 having a higher yield of 4.5 kg compared to V2 which had a yield of 3.9 kg. This suggests that V1 may be a more consistent performer over the two years.

Table 6 Yield Performance Comparison of Varietal Selections across Two Growing Seasons

Variety	Year	Yield (kg)
V1	2021	4
V1	2022	4.5
V2	2021	3.5
V2	2022	3.9

4. DISCUSSIONS

The present study aimed to investigate the effects of different NPK treatments on the growth and yield of canola varieties. The results indicate that treatment T1, which had the highest NPK dose (250:188:75 g/Marla), had the most positive effect on plant growth and yield. These findings are consistent with previous studies, Tayo and Morgan, (1975) regarding *Brassica napus*, where the average weight of 3.28 g/1000 seeds was documented. It was observed that the timely delivery of assimilates to the pod is of paramount importance for seed development and plants with balanced nutrient supplies produced heavier seeds compared to those with no or limited applications of N, P and K, either alone or in combination. This discovery is consistent with the findings of Allen and Morgan. The results revealed that plant height increased linearly with every progressive increase in NPK, which was correlated with the gradual rise in plant height. These findings corroborate previous research by Ayub et al., (2002) and Maqsood et al., (2001). The smallest grain weight (22.60g) was obtained when no fertilizer was applied, which aligns with the observations made by Sharma and Gupta, (1998) that the grain weight of cereals is significantly influenced by NPK fertilizer levels.

The results indicate that the V1 canola variety demonstrated superior performance as compared to the V2 variety. These findings are in agreement with prior research Li et al., (2022) examined the impact of combined nitrogen and potassium fertilizers on crop yield and nitrogen environmental impact. The researchers noted that excessive use of nitrogen fertilizers and depletion of potassium in soil can lead to nutrient imbalances and exacerbate nitrogen loss in intensive crop production. To investigate this issue, the researchers grew rapeseed plants in a 3-year field experiment using two cultivars in the Yangtze River Basin. They found that combined nitrogen and potassium fertilization was necessary to achieve high seed yield and the contribution of nitrogen fertilizer to seed yield depended on the potassium nutritional status. After the application of combined nitrogen and potassium fertilization, the yield of the H9 and Z11 cultivars increased significantly by 153.2%-397.5% and 150.4%-322.9%, respectively, compared to the treatment without the use of nitrogen and potassium fertilizers. The increase was significantly higher than the yield obtained with nitrogen or potassium fertilization alone. In conclusion, this study provides evidence supporting the importance of timely nutrient delivery to achieve optimum seed development and production. Furthermore, the linear relationship between plant height and NPK fertilizers suggests that fertilization can also affect the physical attributes of the plants. These findings can be valuable for crop growers and agronomists in optimizing the production of *Brassica napus* and other crops. Future research could investigate the underlying mechanisms by which nutrient supply affects seed development and plant growth.

The outcomes of this research hold substantial importance for canola producers and growers. The study reveals that the application of NPK fertilizer can play a crucial role in boosting canola growth and yield. Additionally, the selection of appropriate canola variety can significantly impact the yield of the crop. Therefore, while making management decisions, it is crucial for producers to consider both NPK fertilizer and variety selection. Future research may investigate the optimal NPK doses and ratios for different types of canola, as well as the interrelationships between NPK fertilizer and other management strategies. However, it is essential to note that this study was conducted over two consecutive years and may not fully account for the variation in canola growth and yield across multiple growing seasons.

It is noteworthy that there were considerable differences observed between the data collected from the same varieties in 2021 and 2022. These variations may have arisen due to the differences in environmental conditions during the two years. In particular, the 2021 growing season experienced a sudden increase in temperature, resulting in a heat wave that may have adversely impacted the crop and led to negative outcomes in comparison to the data collected in 2022. While the effects of temperature on crop growth and productivity have been studied extensively in the scientific literature, it is important to consider the specific conditions of the experimental setup to draw definitive conclusions. Adverse weather conditions are known to have a detrimental effect on canola, also referred to as oilseed rape, resulting in reduced crop yield. The negative impact of heat stress on yield components during the flowering phase has been extensively studied across various crops. For instance, Guilioni et al., (1997) observed that a brief period of heat stress led to increased floral bud and flower abortion in field pea. In line with this, our own experiment (Secchi et al., 2023) revealed that heat stress resulted in a 5% reduction in the overall seed oil concentration. Moreover, imposing heat stress for a

prolonged period during the early flowering stage resulted in a significant decline of 14% in the seed oil concentration, as determined through controlled conditions.

5. CONCLUSION

In conclusion, the study examined the effects of different NPK doses on the growth and yield of canola plants, as well as the performance of two canola varieties. The findings revealed that treatment T1 with NPK (250:188:75 g/marla) had the most positive effect on yield components, with the highest values for plant height, plant population, number of pods per plant, 1000-grain weight and yield. Based on the data, it has been observed that the Punjab canola variety has produced higher yields in both years 2021 and 2022. Specifically, on recommended doses of NPK (250:188:75 g/marla), Punjab canola produced 4 kg yield per marla in 2021 and 4.5 kg yield per Marla in 2022. In comparison, the Faisal canola V2 variety produced 3.5 kg yield in 2021 and 3.9 kg yield in 2022 on the same recommended NPK doses. Therefore, it is recommended to farmers that they use the Punjab canola variety with the recommended NPK doses (250:188:75 g/Marla) for optimal yield. It is important to note that these calculations and experiments were conducted on a single Marla. If we extrapolate these results to one hectare, farmers could expect to obtain a yield of 1750-1800 kg or 44-45 mund per hectare with the recommended NPK doses (NPK 100:75:30 kg/ha-1). Furthermore, this study found that Punjab canola is best suited for the Layyah zone, especially for the Shahpur Durata soil and climate conditions. Overall, these observations suggest that the use of recommended NPK doses with the Punjab canola variety can lead to higher yields for farmers in this region.

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Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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